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<p>(54) Title: METHODS FOR TREATMENT OF ALLERGIC ASTHMA</p> <p>(57) Abstract</p> <p>Methods of treatment of allergic asthma with IgE antagonists, including anti-IgE antibodies, IgE variants, peptide antagonists, peptidomimetics and other small molecules, are described.</p> <div data-bbox="987 1163 1365 1927"><p>PLACEBO - Allergen adjusted by Diluent: Day -1(-4) (dashed) & Day 0(0) (solid)</p><p>FEV1 % OF BASELINE</p><p>MIN. AFTER CHALLENGE</p><p>ESS - Allergen adjusted by Diluent: Day -1(-4) (dashed) & Day 0(0) (solid)</p><p>FEV1 % OF BASELINE</p><p>MIN. AFTER CHALLENGE</p></div>		

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METHODS FOR TREATMENT OF ALLERGIC ASTHMA**FIELD OF THE INVENTION**

This invention relates to methods of treatment of allergic asthma with IgE antagonists, including anti-IgE antibodies.

BACKGROUND OF THE INVENTION

5 Asthma is characterized by three components: airways inflammation; airways obstruction, which is reversible; and increased sensitivity, referred to as hyperreactivity. Obstruction to airflow is measured by a decrement in forced expired volume in one second (FEV₁) which is obtained by comparison to baseline spirometry. Hyperreactivity of the airways is recognized by decreases in FEV₁ in response to very low levels
10 of histamine or methacholine. Hyperreactivity may be exacerbated by exposure of the airways to allergen.

People with allergic asthma who inhale an aerosolized allergen to which they are sensitive develop immediate "early asthmatic response" (EAR), and often delayed "late asthmatic response" (LAR), as measured by FEV₁ (Cockcroft et al. Clin. Allergy 7:503-13 (1977); Hargreave et al. J. Allergy Clin. Immunol. 83:525-7 (1989)). The EAR results from mast cell degranulation provoked by cross linking of an antigen with mast cell-bound IgE. Preformed mediators (such as histamine and tryptase) and newly formed lipid mediators (such as
15 prostaglandins and leukotrienes) released from mast cells cause bronchoconstriction, mucus hypersecretion, and changes in vascular permeability (Fick et al. J. Appl. Phys. 63:1147-55 (1987)). EAR recovery occurs within 30-60 minutes. The LAR is associated with more pronounced airway inflammation characterized by eosinophilic and neutrophilic infiltration of the mucosa, more prolonged changes in bronchovascular permeability, and
20 increased bronchial reactivity to non-specific stimuli (Hargreave et al., supra; Fick et al. supra; Diaz et al. Am. Rev. Respir. Dis. 139:1383-9 (1989); Fahy et al. J. Allergy Clin. Immunol. 93:1031-9 (1994)). The pathophysiologic relationship between LAR and EAR remains unclear, but the LAR airway changes may be caused by mast cell mediators (including cytokines) released during EAR. The activities of these mast cell mediators include chemoattraction of inflammatory cells to the airway mucosa and the induction of more
25 prolonged changes in vascular permeability in the airway mucosa (Fick et al. Am. Rev. Respir. Dis. 135:1204-9 (1987)).

IgE is thought to be the central effector antibody in the EAR to inhaled allergen in people with allergic asthma, although its role in the LAR is unclear. Chronic allergic asthma may result from continual degranulation of airway mast cells in response to continued exposure to perennial allergens (i.e., the house dust mite, dog
30 dander, and cat hair). This hypothesis is supported by studies which demonstrate a reduction in asthma symptoms and in bronchial hyperreactivity in subjects who reduce their environmental exposure to aeroallergens (Platts-Mills et al. Lancet ii:675-8 (1982); Murray et al. Pediatrics 71:418-22 (1983)).

Airway hyperreactivity reactions can be induced in the laboratory by exposing subjects with allergic asthma to nebulized solutions of allergen extract, the concentration of which can be determined by airway
35 hyperreactivity to methacholine and skin test reactivity to the same allergen. This procedure is known as experimental aerosolized allergen challenge or bronchial provocation. Bronchial provocation is a useful and relevant model for the study of anti-asthma medications (Cockcroft et al. J. Allergy Clin. Immunol. 79:734-40 (1987); Cresciolli et al. Ann. Allergy 66:245-51 (1991); Ward et al. Am. Rev. Respir. Dis. 147:518-23 (1993)).

For example, it is known that beta agonists inhibit the EAR but not the LAR to allergen, and that a single dose of inhaled steroid inhibits the LAR but not the EAR (Cockcroft et al., J. Allergy Clin. Immunol. 79:734-40 (1987)). Theophylline and disodium cromoglycate attenuate both the EAR and LAR responses to allergen (Cresciolli et al., supra; Ward et al. supra). Most drugs with proven efficacy in asthma management have been shown to attenuate airway responses to inhaled antigens administered in bronchial provocation. If airway mast cell-bound IgE is central to the airway response to inhaled allergen, then decreasing or eliminating circulating and mast cell bound IgE may result in significant attenuation of the EAR and possibly also the LAR to inhaled aeroallergens.

The concept of using anti-IgE antibodies as a treatment for allergy has been widely disclosed in the scientific literature. A few representative examples are as follows. Baniyash and Eshhar (European Journal of Immunology 14:799-807 (1984)) demonstrated that an anti-IgE monoclonal antibody could specifically block passive cutaneous anaphylaxis reaction when injected intradermally before challenging with the antigen; U.S. 4,714,759 discloses a product and process for treating allergy, using an antibody specific for IgE; and Rup and Kahn (International Archives Allergy and Applied Immunology, 89:387-393 (1989) discuss the prevention of the development of allergic responses with monoclonal antibodies which block mast cell-IgE sensitization.

Anti-IgE antibodies which block the binding of IgE to its receptor on basophils and which fail to bind to IgE bound to the receptor, thereby avoiding histamine release are disclosed, for example, by Rup and Kahn (supra), by Baniyash et al. (Molecular Immunology 25:705-711, 1988), and by Hook et al. (Federation of American Societies for Experimental Biology, 71st Annual Meeting, Abstract #6008, 1987).

Antagonists of IgE in the form of receptors, anti-IgE antibodies, binding factors, or fragments thereof have been disclosed in the art. For example, U.S. 4,962,035 discloses DNA encoding the alpha-subunit of the mast cell IgE receptor or an IgE binding fragment thereof. Hook et al. (Federation Proceedings Vol. 40, No. 3, Abstract #4177) disclose monoclonal antibodies, of which one type is anti-idiotypic, a second type binds to common IgE determinants, and a third type is directed towards determinants hidden when IgE is on the basophil surface.

U.S. 4,940,782 discloses monoclonal antibodies which react with free IgE and thereby inhibit IgE binding to mast cells, and react with IgE when it is bound to the B-cell FcE receptor, but do not bind with IgE when it is bound to the mast cell FcE receptor, nor block the binding of IgE to the B-cell receptor.

U.S. 4,946,788 discloses a purified IgE binding factor and fragments thereof, and monoclonal antibodies which react with IgE binding factor and lymphocyte cellular receptors for IgE, and derivatives thereof.

U.S. 5,091,313 discloses antigenic epitopes associated with the extracellular segment of the domain which anchors immunoglobulins to the B cell membrane. The epitopes recognized are present on IgE-bearing B cells but not basophils or in the secreted, soluble form of IgE. U.S. 5,252,467 discloses a method for producing antibodies specific for such antigenic epitopes. U.S. 5,231,026 discloses DNA encoding murine-human antibodies specific for such antigenic epitopes.

U.S. 4,714,759 discloses an immunotoxin in the form of an antibody or an antibody fragment coupled to a toxin to treat allergy.

Presta et al. (*J. Immunol.* 151:2623-2632 (1993)) disclose a humanized anti-IgE antibody that prevents the binding of free IgE to FcεRI but does not bind to FcεRI-bound IgE. Copending WO93/04173 discloses polypeptides which bind differentially to the high- and low-affinity IgE receptors.

U.S. 5,428,133 discloses anti-IgE antibodies as a therapy for allergy, especially antibodies which bind to IgE on B cells, but not IgE on basophils. This publication mentions the possibility of treating asthma with such antibodies. U.S. 5,422,258 discloses a method for making such antibodies.

Tepper et al. ("The Role of Mast cells and IgE in Murine Asthma", presented at "Asthma Theory to Treatment", July 15-17, 1995) disclose that neither mast cells nor IgE greatly influence the anaphylaxis, airway hyperreactivity, or airway inflammation in a murine model of asthma.

10

SUMMARY OF THE INVENTION

One embodiment of the invention is a method of treatment of allergic asthma in a patient comprising administering to the patient a maintenance dose of an IgE antagonist and, optionally, a loading dose of the IgE antagonist.

A further embodiment of the invention is a method for treating allergic asthma in a patient comprising administering to the patient a dose of IgE antagonist averaging about 0.001 to 0.01 mg/kg/week IgE antagonist for every IU/ml baseline IgE in the patient's serum.

A further embodiment of the invention is a method of reducing the late asthmatic response in a patient comprising administering to the patient a maintenance dose of an IgE antagonist and, optionally, a loading dose of the IgE antagonist.

A further embodiment of the invention is a method of reducing the late asthmatic response in a patient comprising administering to the patient a dose of IgE antagonist averaging about 0.001 to 0.01 mg/kg/week IgE antagonist for every IU/ml baseline IgE in the patient's serum.

A further embodiment of the invention is a method of reducing the early asthmatic response in a patient comprising administering to the patient a maintenance dose of an IgE antagonist and, optionally, a loading dose of the IgE antagonist.

A further embodiment of the invention is a method of reducing the early asthmatic response in a patient comprising administering to the patient a dose of IgE antagonist averaging about 0.001 to 0.01 mg/kg/week IgE antagonist for every IU/ml baseline IgE in the patient's serum.

A further embodiment of the invention is a method of reducing bronchial hyperreactivity in a patient comprising administering to the patient a maintenance dose of an IgE antagonist and, optionally, a loading dose of the IgE antagonist.

A further embodiment of the invention is a method of reducing bronchial hyperreactivity in a patient comprising administering to the patient a dose of IgE antagonist averaging about 0.001 to 0.01 mg/kg/week IgE antagonist for every IU/ml baseline IgE in the patient's serum.

A further embodiment of the invention is a method of reducing skin reactivity in a patient comprising administering to the patient a maintenance dose of an IgE antagonist and, optionally, a loading dose of the IgE antagonist.

A further embodiment of the invention is a method of reducing skin reactivity in a patient comprising administering to the patient a dose of IgE antagonist averaging about 0.001 to 0.01 mg/kg/week IgE antagonist for every IU/ml baseline IgE in the patient's serum.

5 A further embodiment of the invention is a method of reducing lung inflammation in a patient comprising administering to the patient a maintenance dose of an IgE antagonist and, optionally, a loading dose of the IgE antagonist.

A further embodiment of the invention is a method of reducing lung inflammation in a patient comprising administering to the patient a dose of IgE antagonist averaging about 0.001 to 0.01 mg/kg/week IgE antagonist for every IU/ml baseline IgE in the patient's serum.

10 BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graph depicting the percent change in FEV_1 from baseline in an allergen bronchial challenge in anti-IgE antibody treated patients and patients receiving placebo in the U.S. dosing protocol.

Figures 2 (U.S.) and 3 (Canada) depict the results from methacholine bronchial challenge in anti-IgE antibody treated patients and patients receiving placebo.

15 Figures 4 (U.S.) and 5 (Canada) depict the change from baseline in total symptoms scores in anti-IgE antibody treated patients and patients receiving placebo.

Figures 6 (U.S.) and 7 (Canada) depict endpoint titration skin testing for allergens in patients receiving placebo or anti-IgE antibody.

DETAILED DESCRIPTION OF THE INVENTION

20 A. DEFINITIONS

The term "asthma" as used herein refers to a lung disease characterized by airway obstruction that is reversible (although not entirely in some patients) either spontaneously or with treatment, airway inflammation, and increased airway responsiveness to a variety of stimuli. "Allergic asthma" as used herein refers to an asthmatic response to inhalation of an antigen to which the patient is sensitive.

25 The term "early asthmatic response" (EAR) as used herein refers to an asthmatic response to an antigen within about two hours of exposure. The term "late asthmatic response" (LAR) as used herein refers to an asthmatic response to an antigen within about two to eight hours after exposure.

The term "IgE antagonist" as used herein refers to a substance which inhibits the biological activity of IgE. Such antagonists include but are not limited to anti-IgE antibodies, IgE receptors, anti-IgE receptor antibodies, 30 variants of IgE antibodies, ligands for the IgE receptors, and fragments thereof. Antibody antagonists may be of the IgA, IgD, IgG, IgE, or IgM class. Variant IgE antibodies typically have amino acid substitutions or deletions at one or more amino acid residues. Ligands for IgE receptors include but are not limited to IgE and anti-receptor antibodies, and fragments thereof capable of binding to the receptors, including amino acid substitution and deletion variants, and cyclized variants.

35 In general, in some embodiments of the invention, IgE antagonists act by blocking the binding of IgE to its receptors on B cells, mast cells, or basophils, either by blocking the binding site on the IgE molecule or blocking its receptors. Additionally, in some embodiments of the invention, IgE antagonists act by binding soluble IgE and thereby removing it from circulation. The IgE antagonists of the invention can also act by binding to IgE on B cells, thereby eliminating clonal populations of B cells. The IgE antagonists of the instant

invention can also act by inhibiting IgE production. Preferably, the IgE antagonists of the instant invention do not result in histamine release from mast cells or basophils.

The term "therapeutic amount" as used herein denotes an amount that prevents or ameliorates symptoms of a disorder or responsive pathologic physiological condition.

5 "Polypeptide" as used herein refers generally to peptides and proteins having at least about two amino acids.

The term "free IgE" as used herein refers to IgE not complexed to a binding partner, such as an anti-IgE antibody. The term "total IgE" as used herein refers to the measurement of free IgE and IgE complexed to a binding partner, such as an anti-IgE antibody. The term "baseline IgE" as used herein refers to the level of free
10 IgE in a patient's serum before treatment with an IgE antagonist.

The term "polyol" as used herein denotes a hydrocarbon including at least two hydroxyls bonded to carbon atoms, such as polyethers (e.g. polyethylene glycol), trehalose, and sugar alcohols (such as mannitol).

The term "polyether" as used herein denotes a hydrocarbon containing at least three ether bonds. Polyethers can include other functional groups. Polyethers useful for practicing the invention include
15 polyethylene glycol (PEG).

B. GENERAL METHODS

Polyclonal antibodies to IgE generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of IgE and an adjuvant. It can be useful to conjugate IgE or a fragment containing the target amino acid sequence from the Fc region of IgE to a protein that is immunogenic in the species to be
20 immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl_2 , or $\text{R}^1\text{N}=\text{C}=\text{NR}$, where R and R^1 are different alkyl groups.

Animals ordinarily are immunized against the cells or immunogenic conjugates or derivatives by combining
25 1 mg or 1 μg of IgE with Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of conjugate in Freund's incomplete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later, animals are bled and the serum is assayed for anti-IgE titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with a conjugate of the same IgE, but conjugated to a different protein and/or through a different cross-linking agent.
30 Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum can be used to enhance the immune response.

Monoclonal antibodies are prepared by recovering spleen cells from immunized animals and immortalizing the cells in conventional fashion, e.g. by fusion with myeloma cells or by Epstein-Barr (EB)-virus transformation and screening for clones expressing the desired antibody. The hybridoma technique described originally by
35 Koehler and Milstein, *Eur. J. Immunol.*, 6: 511 (1976) and also described by Hammerling *et al.*, in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens.

The hybrid cell lines can be maintained *in vitro* in cell culture media. The cell lines producing the antibodies can be selected and/or maintained in a composition comprising the continuous cell line in

hypoxanthine-aminopterin thymidine (HAT) medium. In fact, once the hybridoma cell line is established, it can be maintained on a variety of nutritionally adequate media. Moreover, the hybrid cell lines can be stored and preserved in any number of conventional ways, including freezing and storage under liquid nitrogen. Frozen cell lines can be revived and cultured indefinitely with resumed synthesis and secretion of monoclonal antibody.

5 The secreted antibody is recovered from tissue culture supernatant by conventional methods such as precipitation, ion-exchange chromatography, affinity chromatography, or the like. The antibodies described herein are also recovered from hybridoma cell cultures by conventional methods for purification of IgG or IgM, as the case may be, that heretofore have been used to purify these immunoglobulins from pooled plasma, e.g., ethanol or polyethylene glycol precipitation procedures. The purified antibodies are sterile-filtered.

10 While routinely mouse monoclonal antibodies are used, the invention is not so limited; in fact, human antibodies can be used. Such antibodies can be obtained, for example, by using human hybridomas (Cote et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985)). In fact, according to the invention, techniques developed for the production of chimeric antibodies (Cabilly et al., U.S. 4,816,567, Morrison et al., Proc. Natl. Acad. Sci. 81: 6851 (1984); Boulianne et al., Nature 312: 643-646 (1984); Neuberger et al., Nature, 312: 604 (1984); Neuberger et al., Nature 314: 268-270 (1985); Takeda et al., Nature 314: 452 (1985); EP 184,187; EP 171,496; EP 173,494; PCT WO 86/01533; Shaw et al., J. Nat. Canc. Inst. 80: 1553-1559 (1988); Morrison, Science 229: 1202-1207 (1985); Oi et al., BioTechniques, 4: 214 (1986)) by coupling an animal antigen-binding variable domain to a human constant domain can be used; such antibodies are within the scope of this invention. The term "chimeric" antibody is used herein to describe a polypeptide comprising at least the antigen binding portion of an antibody molecule linked to at least part of another protein (typically an immunoglobulin constant domain).

20 In one embodiment, such chimeric antibodies contain about one third rodent (or other non-human species) sequence and thus are capable of eliciting a significant anti-globulin response in humans. For example, in the case of the murine anti-CD3 antibody OKT3, much of the resulting anti-globulin response is directed against the variable region rather than the constant region (Jaffers et al., Transplantation 41: 572-578 (1986)).

25 Humanized antibodies are used to reduce or eliminate any anti-globulin immune response in humans. In practice, humanized antibodies are typically human antibodies in which some amino acid residues from the complementarity determining regions (CDRs), the hypervariable regions in the variable domains which are directly involved with formation of the antigen-binding site, and possibly some amino acids from the framework regions (FRs), the regions of sequence that are somewhat conserved within the variable domains, are substituted by residues from analogous sites in rodent antibodies. The construction of humanized antibodies is described in Riechmann et al., Nature 332: 323-327 (1988), Queen et al., Proc. Natl. Acad. Sci. USA 86: 10029-10033 (1989), Co et al., Proc. Natl. Acad. Sci. USA 88: 2869-2873 (1991), Gorman et al., Proc. Natl. Acad. Sci. 88: 4181-4185 (1991), Daugherty et al., Nucleic Acids Res. 19: 2471-2476 (1991), Brown et al., Proc. Natl. Acad. Sci. USA 88: 2663-2667 (1991), Junghans et al., Cancer Res. 50: 1495-1502 (1990), Fendly et al., Cancer Res. 50: 1550-1558 (1990) and in PCT applications WO 89/06692 and WO 92/22653.

In some cases, substituting CDRs from rodent antibodies for the human CDRs in human frameworks is sufficient to transfer high antigen binding affinity (Jones et al., Nature 321: 522-525 (1986); Verhoeven et al.,

Science 239: 1534-1536 (1988)) whereas in other cases it is necessary to additionally replace one (Riechmann et al., supra) or several (Queen et al., supra) FR residues. See also Co et al., supra.

The invention also encompasses the use of human antibodies produced in transgenic animals. In this system, DNA encoding the antibody of interest is isolated and stably incorporated into the germ line of an animal host. The antibody is produced by the animal and harvested from the animal's blood or other body fluid. Alternatively, a cell line that expresses the desired antibody can be isolated from the animal host and used to produce the antibody in vitro, and the antibody can be harvested from the cell culture by standard methods.

Anti-IgE antibody fragments can also be used in the methods of the invention. Any fragment of an anti-IgE antibody capable of blocking or disrupting IgE interaction with its receptor is suitable for use herein.

Suitable anti-IgE antibody fragments can be obtained by screening combinatorial variable domain libraries for DNA capable of expressing the desired antibody fragments. These techniques for creating recombinant DNA versions of the antigen-binding regions of antibody molecules which bypass the generation of monoclonal antibodies, are encompassed within the practice of this invention. One typically extracts antibody-specific messenger RNA molecules from immune system cells taken from an immunized animal, transcribes these into complementary DNA (cDNA), and clones the cDNA into a bacterial expression system. "Phage display" libraries are an example of such techniques. One can rapidly generate and screen great numbers of candidates for those that bind the antigen of interest. Such IgE-binding molecules are specifically encompassed within the term "antibody" as it is defined, discussed, and claimed herein.

In a further embodiment of the invention, soluble IgE receptor can be used as the IgE antagonist. Soluble receptors suitable for use herein include, for example, molecules comprising the IgE binding site in the extracellular domain (exodomain) of the FcεRI α chain. The α chain of FcεRI can be genetically modified such that the exodomain is secreted as a soluble protein in a recombinant expression system according to the method of Blank et al., J. Biol. Chem., 266: 2639-2646 (1991) or Qu et al., J. Exp. Med., 167: 1195.

The invention also encompasses the use of IgE-binding peptides in addition to anti-IgE antibodies and soluble receptor. Any IgE-binding peptide capable of disrupting or blocking the interaction between IgE and its receptors is suitable for use herein.

In addition to IgE antagonists which interfere with IgE/receptor interaction by binding to IgE, such as anti-IgE antibodies, fragments thereof, soluble IgE receptor and other IgE-binding peptides described above, the invention encompasses the use of IgE antagonists which disrupt IgE/receptor interaction by competing with IgE for binding to its receptor, thereby lowering the available IgE receptor.

IgE variants are an example of a receptor-binding competitor that is suitable for use in the methods of the invention. IgE variants are forms of IgE possessing an alteration, such as an amino acid substitution or substitutions and/or an amino acid deletion or deletions, wherein the altered IgE molecule is capable of competing with IgE for binding to its receptors.

Fragments of IgE variants are also suitable for use herein. Any fragment of an IgE variant capable of competing with IgE for binding to its receptors can be used in the methods of the invention.

The invention also encompasses the use of IgE receptor-binding peptides in addition to IgE variants and fragments thereof. Any IgE receptor-binding peptide capable of disrupting or blocking the interaction between IgE and its receptors is suitable for use herein.

The amount of IgE antagonist delivered to the patient to be used in therapy will be formulated and dosages established in a fashion consistent with good medical practice taking into account the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Similarly, the dose of the IgE antagonist administered will be dependent upon the properties of the IgE antagonist employed, e.g. its binding activity and in vivo plasma half-life, the concentration of the IgE antagonist in the formulation, the administration route, the site and rate of dosage, the clinical tolerance of the patient involved, the pathological condition afflicting the patient and the like, as is well within the skill of the physician.

Typically IgE antagonists are administered by intramuscular, intravenous, intrabronchial, intraperitoneal, subcutaneous or other suitable routes. The antagonists can be administered before and/or after the onset of symptoms. In general, a "loading" dose of an IgE antagonist is useful to obtain a rapid and sustained decrease in free IgE. A loading dose is typically a first dose of IgE antagonist that is greater than a subsequent or "maintenance" dose of IgE antagonist. However, patients can be loaded in other ways. For example, patients can be loaded by administering a dose of antagonist that is greater than or equal to the same mg/kg amount as the maintenance dose, but increasing the frequency of administration in a "loading regimen". Thus, for example, if the maintenance dose is 1 mg/kg biweekly, the patient can be loaded by administering 1 mg/kg weekly for two or more weeks in a row, then administering the maintenance dose of 1 mg/kg biweekly. Furthermore, patients can be loaded during a course of treatment with a maintenance dose of IgE antagonist by administering larger or more frequent doses than the maintenance dose. The term "loading dose" is intended as used herein to include such single loading doses, multiple loading doses, loading regimens, and combinations thereof.

A sustained decrease in free IgE can be obtained by administration of a maintenance dose of the antagonist. Maintenance doses are delivered with a frequency of about every day to about every 90 days, more preferably weekly to biweekly, depending on the severity of the patient's symptoms, the concentration and in vivo properties of antagonist delivered, and the formulation of the antagonist. For example, slow release formulations can allow less frequent administration. Maintenance doses can be adjusted upwards or downwards over time, depending on the response of the patient.

Thus, for example, in one embodiment of the invention, the dose of IgE antagonist is sufficient to reduce free IgE in the patient's serum to less than about 40 ng/ml.

In a further dosing strategy, about 0.05 to 10 mg/kg, more preferably about 0.1 to 1 mg/kg, most preferably about 0.5 mg/kg IgE antagonist can be administered on a weekly basis to a patient having about 40-200 IU/ml baseline IgE. In another dosing strategy for individuals with higher baseline IgE, patients are preferably "loaded" with about 1 to about 10 mg/kg, more preferably about 1 to about 5 mg/kg, most preferably about 2 mg/kg, IgE antagonist, followed by weekly or biweekly administration of about 0.1 to about 10 mg/kg, most preferably about 1 mg/kg.

In a further dosing strategy, a maintenance dose of IgE antagonist averaging about 0.0005 to 0.05 mg/kg/week for every IU/ml baseline IgE, more preferably 0.001 to about 0.01 mg/kg/week for every IU/ml baseline IgE is used. This maintenance regimen can follow an initial loading dose of about 1 to 10 mg/kg, more preferably about 1 to 5 mg/kg IgE antagonist.

In a further embodiment of the invention, sufficient IgE antagonist is provided through the maintenance dose, and, optionally, the loading dose, to achieve about a 1 to 20 fold, preferably about 3 to 5 fold, most preferably about a 5 fold greater serum concentration than total serum IgE concentration in the patient.

IgE levels are typically assayed by standard ELISA techniques well known in the art. Total serum IgE can be measured by commercially available assays, such as Abbott Laboratories' Total IgE assay. Free IgE, e.g., IgE not bound to antibody can be measured by a capture type assay in which, for example, IgE receptor is bound to a solid support. IgE complexed to an anti-IgE antibody which binds at or near the site on IgE which binds to the receptor will be blocked from binding the receptor, and thus only free or unbound IgE can react with the receptor bound to the solid support in this assay. An anti-IgE antibody which recognizes IgE even when the IgE is bound to its receptor can be used to detect the IgE captured by the receptor on the solid support. This anti-IgE antibody can be labeled with any of a variety of reporter systems, such as alkaline phosphatase, etc.

It is envisioned that injections (intravenous, intramuscular or subcutaneous) will be the primary route for therapeutic administration of the IgE antagonist of this invention, although delivery through catheter or other surgical tubing is also used. Alternative routes include suspensions, tablets, capsules and the like for oral administration, commercially available nebulizers for liquid formulations, and inhalation of lyophilized or aerosolized microcapsules, and suppositories for rectal or vaginal administration. Liquid formulations can be utilized after reconstitution from powder formulations.

Additional pharmaceutical methods may be employed to control the duration of action of the antagonists of this invention. The antagonists also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Osol, A., ed., 1980).

In general, the formulations of the subject invention can contain other components in amounts not detracting from the preparation of stable forms and in amounts suitable for effective, safe pharmaceutical administration. For example, other pharmaceutically acceptable excipients well known to those skilled in the art can form a part of the subject compositions. These include, for example, salts, various bulking agents, additional buffering agents, chelating agents, antioxidants, cosolvents and the like; specific examples of these include tris-(hydroxymethyl)aminomethane salts ("Tris buffer"), and disodium edetate.

In one embodiment of the invention, IgE antagonist formulations comprise a buffer, a salt, optionally, a polyol, and optionally, a preservative.

One exemplary formulation of the invention is a liquid formulation of about 1-100 mg/ml IgE antagonist in 10 mM acetate buffer, pH 5.0-6.5, 100-200 mM sodium chloride, and about 0.01% polysorbate 20, more preferably about 5 mg/ml IgE antagonist in 10 mM acetate buffer, pH 5.2, 142 mM sodium chloride, and 0.01% polysorbate 20. In other embodiments of the invention, the formulation may be freeze-dried and reconstituted for administration. For example, anti-IgE antibody can be formulated at about 25 mg/ml in 5 mM histidine, pH 6.0, and 88 mM sucrose, freeze-dried, and reconstituted in water to 100 mg/ml antibody for administration. Mixed sugars can also be used, such as a combination of sucrose and mannitol, etc.

In general, unless otherwise specified, the abbreviations used for the designation of amino acids and the protective groups used therefor are based on recommendations of the IUPAC-IUB Commission of Biochemical Nomenclature (Biochemistry, 11:1726-1732 (1972)). The nomenclature used to define compounds of the invention is that specified by the IUPAC, published in European Journal of Biochemistry 138:9-37 (1984).

5 Therapy of allergic asthma can be combined with other known therapies for allergy and/or asthma, including anti-histamines, theophylline, salbutamol, beclomethasone dipropionate, sodium cromoglycate, steroids, anti-inflammatory agents, etc.

Further details of the invention can be found in the following examples, which further define the scope of the invention. All references cited herein are expressly incorporated by reference in their entireties.

10

EXPERIMENTAL RESULTS

The objects of this randomized, double blind, placebo-controlled, multi-center Phase II clinical study were to determine if an IgE antagonist in the form of an anti-IgE antibody inhibits the EAR and/or the LAR to an inhaled aeroallergen in asthmatic patients. Additional parameters examined included inhibition of the increase in bronchial reactivity, inhibition of the increase in biologic markers of inflammation, and decrease in asthma
15 symptoms in response to treatment with an anti-IgE antibody. The anti-IgE antibody E25 used in this study was the humanized version of monoclonal antibody MaE11 described in Presta et al., supra.

Two dosing protocols were used (denoted U.S. and Canada). In both protocols, the subjects underwent an initial (control) allergen diluent provocation challenge and then two allergen bronchial challenges separated by approximately eight weeks of study drug. 10 U.S. and 9 Canada subjects received anti-IgE antibody E25 and
20 9 U.S. and 9 Canada subjects received placebo therapy. The allergens used for the study were house dust mite, cat hair, or grass. The allergen used for each individual patient was the one that elicited the most positive response in that patient's allergen skin prick challenge test. On Day 0, the day after the first (baseline) allergen bronchial challenge, U.S. patients received 0.5 mg/kg E25 or placebo equivalent intravenously. U.S. patients subsequently received 0.5 mg/kg E25 or placebo equivalent intravenously weekly. Canada subjects received 2.0
25 mg/kg E25 or placebo equivalent on Day 0, 1.0 mg/kg E25 or placebo equivalent on days 7 and 14, and 1.0 mg/kg biweekly thereafter. In both the Canada and U.S. studies, one patient receiving E25 withdrew because of an asthma attack.

The airway effects of inhaled antigen solution was evaluated in three ways in the U.S. protocol and two ways in the Canada protocol. In the U.S. protocol, first, allergen-induced reductions in airflow during the EAR and LAR were recorded by measuring changes from baseline in FEV₁. Second, LAR changes in bronchial
30 reactivity to methacholine were measured. Third, changes in inflammatory markers (eosinophil and neutrophil percent and number, eosinophil cationic protein, myeloperoxidase (MPO) and tryptase) in induced sputum were measured during the LAR.

In the Canada protocol, changes in the amount of aeroallergen required to provoke a greater than or equal to 15% decrease in FEV₁ (PD₁₅) were quantitated. Second, changes in the methacholine concentration required to induce a greater than or equal to 20% fall in FEV₁ (PD₂₀ Mch) were assessed a day prior to the first and last
35 aeroallergen bronchoprovocation and on Day 42.

In the U.S., the primary efficacy variable was the change in FEV₁ measured within one hour of allergen challenge (EAR) between Day -1 and Day 63. The baseline was defined as the observed difference in the percent

change from prechallenge levels in FEV₁ response between the Day-6 allergen diluent challenge and the Day-1 allergen challenge. Follow-up was defined as the difference between the Day 56 allergen diluent challenge and the Day 63 allergen diluent challenge. In each case, two variables were derived: maximal observed decrease and area under the curve (AUC) as approximated by the trapezoidal rule. Treatment efficacy was based on the
 5 between treatment comparison of the baseline and follow-up of AUC and maximal decrease. Between group differences for the change between Day-1 and Day 63 were assessed by the Wilcoxon Rank sum test.

The LAR was measured in a similar fashion as the primary efficacy variable of change in the FEV₁ (AUC and maximal decrease):

The initial dosing of allergen for inhalation was four doubling doses below that calculated from the
 10 prediction formula: $y = 0.69x + 0.11$, where $y = \log_{10}$ allergen PD₂₀FEV₁ (the dose of allergen that causes a 20% fall in FEV₁) and $x = \log_{10}$ methacholine PD₁₀ (the dose of methacholine that causes a 10% fall in FEV₁) X skin allergen sensitivity (skin sensitivity to allergen is defined as the smallest allergen dilution that gives a wheal 2 mm in diameter). When the dose caused a fall in FEV₁ of 20% or more than no further allergen was delivered. When the dose caused a fall in FEV₁ of less than 10%, then the challenge advanced to the next
 15 doubling step, etc. The FEV₁ was measured at 20, 30, 45, 60, 90, and 120 minutes and at hourly intervals up to 7 hours after inhalation.

Dosing of allergen for the second bronchial challenge commenced at an allergen concentration of four doubling doses more dilute than the dose which caused a 20% fall in FEV₁ during the first challenge. The dosing then proceeded in two-fold more concentrated steps until the FEV₁ fell by 20% or until allergen was
 20 delivered at a concentration one doubling dose higher than that delivered on Day-1.

The results for FEV₁ percent change from baseline in allergen bronchial challenge in the U.S. dosing protocol are shown in tabular form in Tables I and II and in graphic form in Figure 1. The numerical values were adjusted by the diluent challenge. One patient withdrew from the study, reducing the total number enrolled to
 19.

25 These data indicate that this treatment protocol with an anti-IgE antibody effectively reduced the EAR by 43% and the LAR by 82%.

Table I. EAR FEV₁ Percent Change from Baseline in Allergen Challenge

	Placebo	E25
Total Number of Patients	9	9
Maximum Decrease		
30 Before Treatment	30%	25%
After Treatment	34%	16%
Improvement	-15%	37%
p-value	0.05	
Area Under Curve		
35 Before Treatment	1320	1319
After Treatment	1506	752
Improvement	-14%	43%
p-value	0.02	

Table II. LAR FEV₁ percent Change from Baseline in Allergen Challenge

	Placebo	E25
Total Number of Patients	9	9
Maximum Decrease		
Before Treatment	15%	21%
5 After Treatment	15%	5%
Improvement	0%	76%
p-value	0.05	
Area Under Curve		
Before Treatment	2235	4928
10 After Treatment	2759	864
Improvement	-23%	82%
p-value	0.04	

The effect of treatment with anti-IgE antibody was also apparent in the assessment of the concentration of allergen delivered during the second allergen challenge. These results, depicted in Table III, indicate that in 15 7/9 patients receiving E25 (as opposed to 2/9 patients receiving placebo), the dose of allergen necessary to achieve a 20% reduction in FEV₁ was increased by 100%.

Table III. Concentration of Allergen Delivered During the Second Allergen Challenge

	Placebo	E25
Total Number of Patients	9	9
100% Increase	2	7
20 Unchanged	1	1
Reduced by 50%	5	0
Reduced by 75%	0	1
Reduced by 88%	1	0

In Canada, the primary efficacy endpoint was the EAR allergen PC₁₅ concentration (the allergen 25 concentration needed to reach the 15% decrease in EAR FEV₁ after the allergen challenge). Basically, subjects inhaled increasing doubling doses at about 12 minute intervals until an FEV₁ measurement demonstrating a decrease of at least 15% or greater was obtained. The PC₁₅ was calculated using the last concentration of allergen (C2), the second last concentration of allergen (C1), the percent fall FEV₁ after C2 (R2) and the percent fall FEV₁ after C1 (R1) and the formula: $\log_{10} \text{allergen PC}_{15} = 0.3(15-R1)/(R2-R1) + \log_{10} C1$. Baseline was 30 defined as the allergen PC₁₅ concentration on Day-1. The primary efficacy variable was the change of allergen PC₁₅ concentration measured on Day 77. Between group differences for the change between Day-1 and Day 77 were assessed by the Wilcoxon Rank sum test. The changes of log allergen PC₁₅ measured within 1 hour of the allergen challenge on days 27 and 55 from baseline were also be compared between the two groups by the Wilcoxon Rank sum test.

Dosing for the subsequent challenges in the Canada protocol commenced at the same allergen challenge as the preceding challenge. However, if the FEV_1 did not fall by greater than or equal to 15% at the same concentration that caused a greater than or equal to 15% fall during the first challenge, up to three additional doubling doses were delivered until a greater than or equal to 15% decrease was observed.

- 5 The results for the bronchial provocation testing with allergen in the Canada protocol are provided in tabular form in Table IV. The results demonstrate that patients receiving anti-IgE therapy required substantially more antigen (more doubling doses) to decrease their FEV_1 to at least 15% of baseline.

Table IV. PC₁₅ Concentration Change

		Placebo	E25
	Day 27		
	< 1 doubling or no improvement	75%	10%
	> 1 doubling	25%	90%
	> 2 doublings	0%	60%
	> 3 doublings	0%	36%
	Median	<0	2.4 doublings
	p= 0.0001		
10	Day 55		
	< 1 doubling or no improvement	89%	20%
	> 1 doubling	11%	80%
	> 2 doublings	0%	60%
	> 3 doublings	0%	48%
	Median	0	2.9 doublings
	p= 0.0008		
	Day 77		
	< 1 doubling or no improvement	67%	20%
	> 1 doubling	33%	80%
	> 2 doublings	11%	70%
	> 3 doublings	0%	45%
	Median	<0	2.9 doublings
	p= 0.002		

- Figures 2 (U.S.) and 3 (Canada) depict the results from methacholine bronchial challenge in treated and untreated patients. Methacholine was delivered at an initial dose of 0.03 mg/ml and a dose-response curve was constructed by administering serial doubling concentrations of methacholine until the worst FEV_1 maneuver recorded 1 or 3 minutes after methacholine inhalation was less than or equal to 80% of the baseline FEV_1 . The $PC_{20}FEV_1$ (methacholine) was calculated by linear interpolation between the last two points open the dose-response challenge. The results in Figures 2 and 3 indicate that as opposed to patients receiving placebo, a
- 15

higher PC₂₀FEV₁ (methacholine) was observed in patients receiving anti-IgE therapy. Thus, patients receiving anti-IgE antibody had reduced hyperresponsiveness as a result of therapy.

Figures 4 (U.S.) and 5 (Canada) depict the change from baseline in total symptoms scores. The subjects in the study were asked to maintain a daily symptoms diary. The parameters included symptoms of asthma such as shortness of breath, chest tightness, wheezing, cough, and sputum (phlegm/mucus); night-time asthma symptoms such as the number of time patient awoke with asthma, AM peak expiratory flow rate (best of three attempts), number of puffs of beta agonist inhaler in the past 12 hours; and day-time asthma symptoms such as absence from work due to asthma, PM peak expiratory flow rate (best of three attempts), and number of puffs of beta agonist inhaler in the past 12 hours. These symptoms were rated on a scale of 0-10, with 10 being extremely severe. The results in Figures 4 and 5 demonstrate that patients receiving anti-IgE therapy exhibit a trend of reduction in asthma related symptoms as a result of treatment.

Figures 6 (U.S.) and 7 (Canada) depict endpoint titration skin testing for allergens in patients receiving placebo or anti-IgE antibody. Basically, patients were intradermally injected with serial tenfold dilutions of the allergen to which the patients were most reactive (house dust mite, cat hair, or grass) at Day -7 and Day 70 (after completion of treatment) to find the highest dilution that caused a skin reaction of 2 mm or less. The results demonstrate that subjects which received anti-IgE antibody had a substantially reduced reactivity to allergen as a result of treatment.

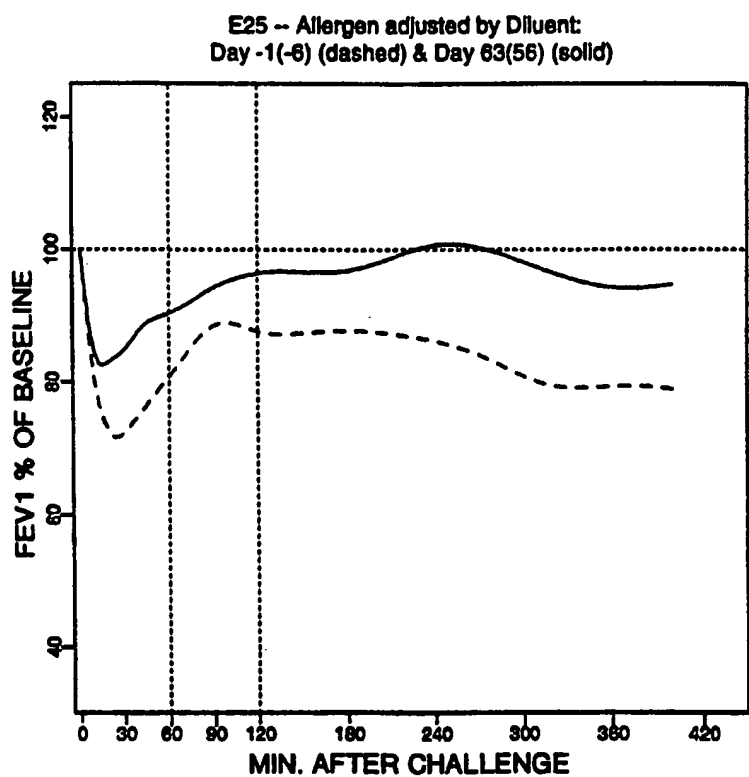
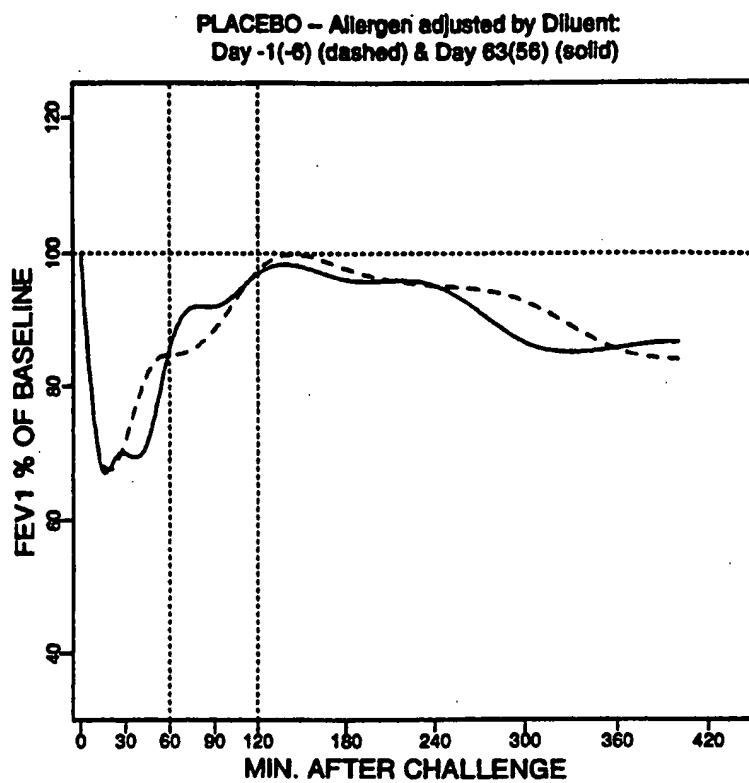
In summary, the results from two dosing protocols demonstrated that treatment with anti-IgE significantly improved early and late asthmatic responses to allergen, non-specific bronchial hyperreactivity, and allergen skin test reactivity. Markers of airway inflammation were also improved.

WE CLAIM:

1. A method of treatment of allergic asthma in a patient comprising administering to the patient a maintenance dose of an IgE antagonist and, optionally, a loading dose of the IgE antagonist.
2. The method of claim 1, wherein the maintenance dose is repeated at intervals of about 1 to about
5 90 days.
3. The method of claim 2, wherein the maintenance dose is repeated weekly.
4. The method of claim 2, wherein the maintenance dose is repeated biweekly.
5. The method of claim 1, wherein the IgE antagonist is an anti-IgE antibody.
6. The method of claim 5, wherein the antibody is chimeric.
- 10 7. The method of claim 6, wherein the antibody is humanized.
8. The method of claim 5, wherein the antibody is a human antibody.
9. The method of claim 1, wherein the antagonist binds to soluble IgE and blocks the binding of IgE to the IgE receptor on basophils.
10. The method of claim 5, wherein the antibody binds to soluble IgE and blocks the binding of IgE
15 to the IgE receptor on basophils.
11. The method of claim 1, wherein the loading dose is administered before onset of asthma symptoms.
12. The method of claim 1, wherein the loading dose is administered after the onset of asthma symptoms.
- 20 13. The method of claim 1, wherein the loading dose is greater than the maintenance dose.
14. The method of claim 1, wherein the antagonist is administered in a formulation comprising a buffer, a salt, optionally, a polyol, and optionally, a preservative.
15. The method of claim 14, wherein the antagonist is freeze-dried, then reconstituted before administration.
- 25 16. The method of claim 1, wherein the maintenance dose, and optionally, the loading dose reduce the concentration of free IgE in the patient's serum to less than about 40 ng/ml.
17. The method of claim 1, wherein the maintenance dose of antagonist is about 0.001 to 0.01 mg/kg/week/baseline IgE IU/ml.
18. The method of claim 1, wherein the maintenance dose, and optionally, the loading dose, results
30 in a total serum concentration of antagonist of about 1 to 10 times greater than the patient's total serum IgE concentration.
19. A method for treating allergic asthma in a patient comprising administering to the patient a dose of IgE antagonist averaging about 0.001 to 0.01 mg/kg/week IgE antagonist for every IU/ml baseline IgE in the patient's serum.
- 35 20. A method of reducing the late asthmatic response in a patient comprising administering to the patient a maintenance dose of an IgE antagonist and, optionally, a loading dose of the IgE antagonist.
21. The method of claim 20, wherein the maintenance dose, and optionally, the loading dose reduce the concentration of free IgE in the patient's serum to less than about 40 ng/ml.

22. The method of claim 20, wherein the maintenance dose, and optionally, the loading dose, results in a total serum concentration of antagonist of about 1 to 10 times greater than the patient's total serum IgE concentration.
23. A method of reducing the late asthmatic response in a patient comprising administering to the patient a dose of IgE antagonist averaging about 0.001 to 0.01 mg/kg/week IgE antagonist for every IU/ml baseline IgE in the patient's serum.
24. A method of reducing the early asthmatic response in a patient comprising administering to the patient a maintenance dose of an IgE antagonist and, optionally, a loading dose of the IgE antagonist.
25. The method of claim 24, wherein the maintenance dose, and optionally, the loading dose reduce the concentration of free IgE in the patient's serum to less than about 40 ng/ml.
26. The method of claim 24, wherein the maintenance dose, and optionally, the loading dose, results in a total serum concentration of antagonist of about 1 to 10 times greater than the patient's total serum IgE concentration.
27. A method of reducing the early asthmatic response in a patient comprising administering to the patient a dose of IgE antagonist averaging about 0.001 to 0.01 mg/kg/week IgE antagonist for every IU/ml baseline IgE in the patient's serum.
28. A method of reducing bronchial hyperreactivity in a patient comprising administering to the patient a maintenance dose of an IgE antagonist and, optionally, a loading dose of the IgE antagonist.
29. The method of claim 28, wherein the maintenance dose, and optionally, the loading dose reduce the concentration of free IgE in the patient's serum to less than about 40 ng/ml.
30. The method of claim 28, wherein the maintenance dose, and optionally, the loading dose, results in a total serum concentration of antagonist of about 1 to 10 times greater than the patient's total serum IgE concentration.
31. A method of reducing bronchial hyperreactivity in a patient comprising administering to the patient a dose of IgE antagonist averaging about 0.001 to 0.01 mg/kg/week IgE antagonist for every IU/ml baseline IgE in the patient's serum.
32. A method of reducing skin reactivity in a patient comprising administering to the patient a maintenance dose of an IgE antagonist and, optionally, a loading dose of the IgE antagonist.
33. The method of claim 32, wherein the maintenance dose, and optionally, the loading dose reduce the concentration of free IgE in the patient's serum to less than about 40 ng/ml.
34. The method of claim 32, wherein the maintenance dose, and optionally, the loading dose, results in a total serum concentration of antagonist of about 1 to 10 times greater than the patient's total serum IgE concentration.
35. A method of reducing skin reactivity in a patient comprising administering to the patient a dose of IgE antagonist averaging about 0.001 to 0.01 mg/kg/week IgE antagonist for every IU/ml baseline IgE in the patient's serum.
36. A method of reducing lung inflammation in a patient comprising administering to the patient a maintenance dose of an IgE antagonist and, optionally, a loading dose of the IgE antagonist..

37. The method of claim 36, wherein the maintenance dose, and optionally, the loading dose reduce the concentration of free IgE in the patient's serum to less than about 40 ng/ml.
38. The method of claim 36, wherein the maintenance dose, and optionally, the loading dose, results in a total serum concentration of antagonist of about 1 to 10 times greater than the patient's total serum IgE concentration.
39. A method of reducing lung inflammation in a patient comprising administering to the patient a dose of IgE antagonist averaging about 0.001 to 0.01 mg/kg/week IgE antagonist for every IU/ml baseline IgE in the patient's serum.
40. The use of an IgE antagonist in the preparation of a pharmaceutical composition for the treatment of allergic asthma comprising a maintenance dose, and optionally, a loading dose of an IgE antagonist.
41. The use of claim 40 for reducing the late asthmatic response in a patient.
42. The use of claim 40 for reducing the early asthmatic response in a patient.
43. The use of an IgE antagonist in the preparation of a pharmaceutical composition for reducing bronchial hyperreactivity, skin reactivity, or inflammation in a patient, comprising a maintenance dose, and optionally, a loading dose of an IgE antagonist.



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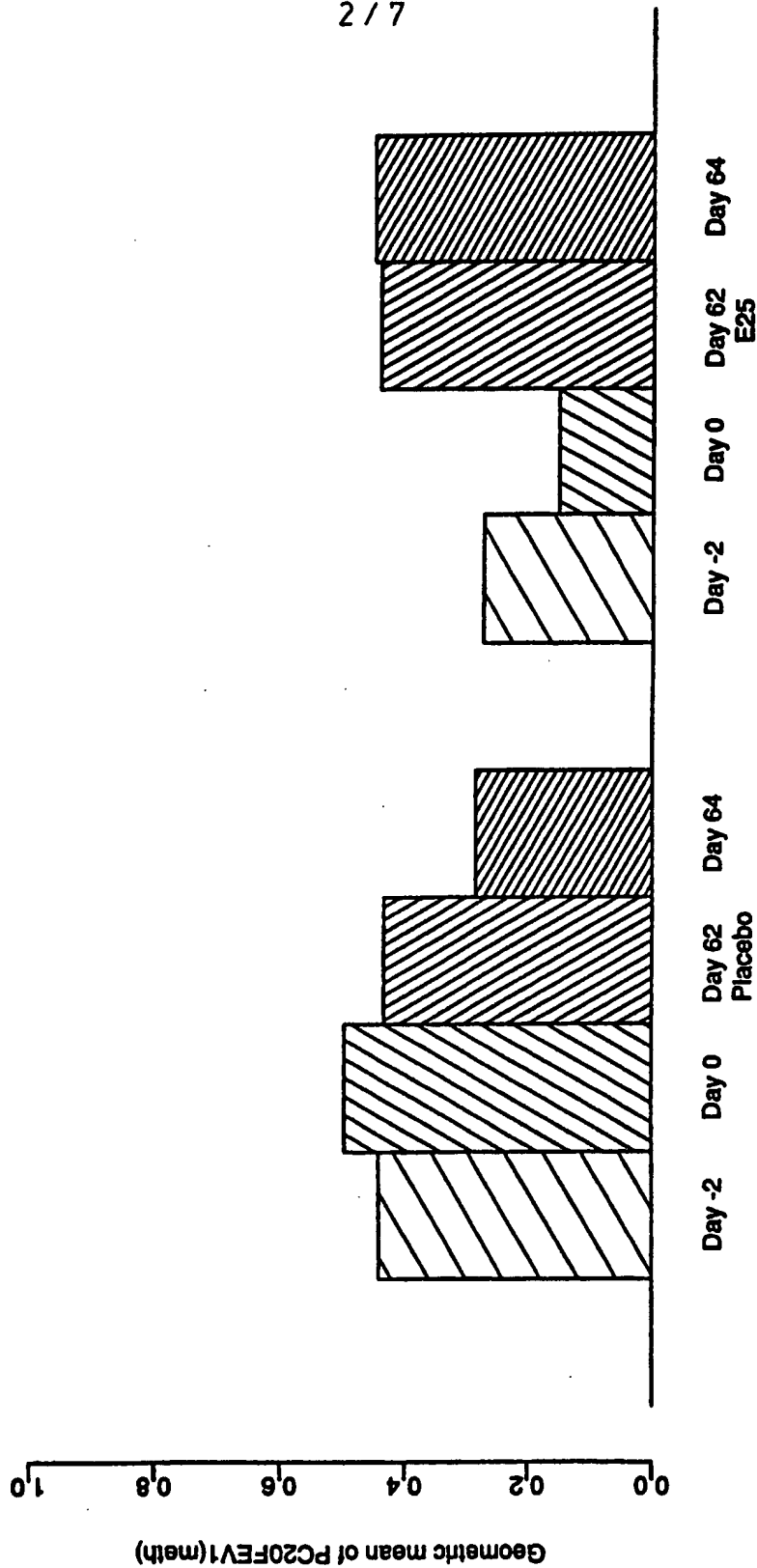


FIG.2

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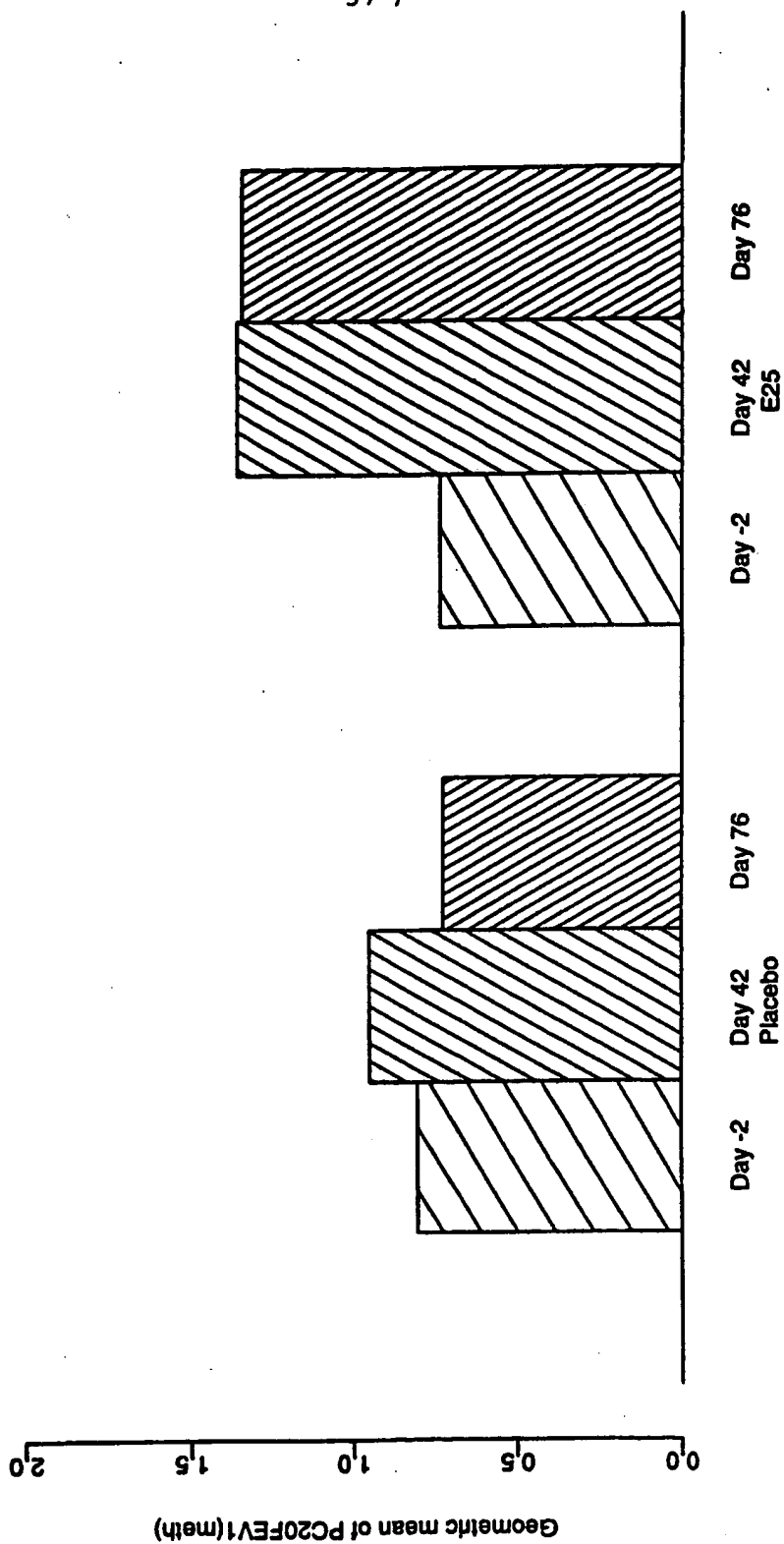


FIG.3

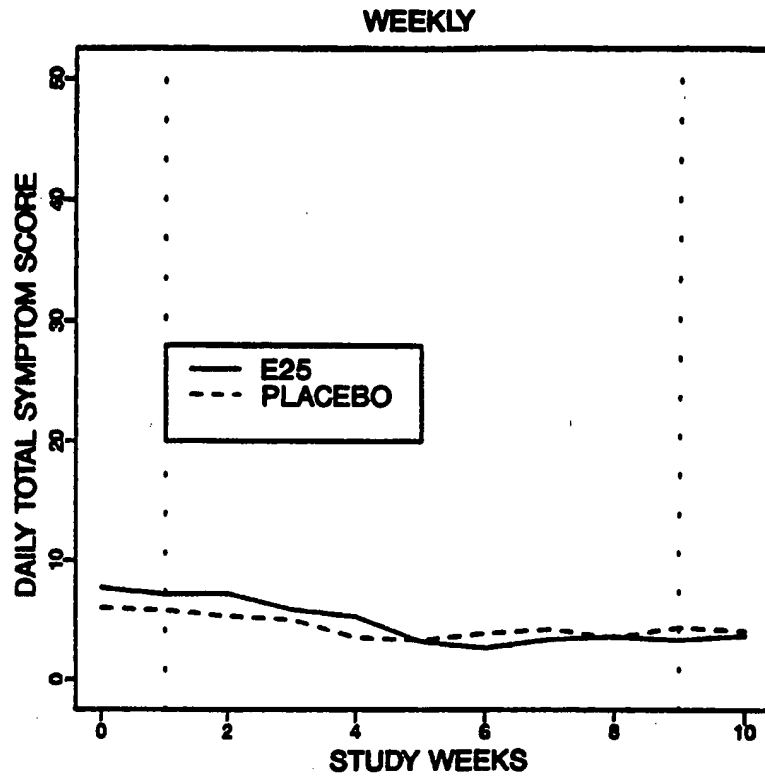


FIG.4A

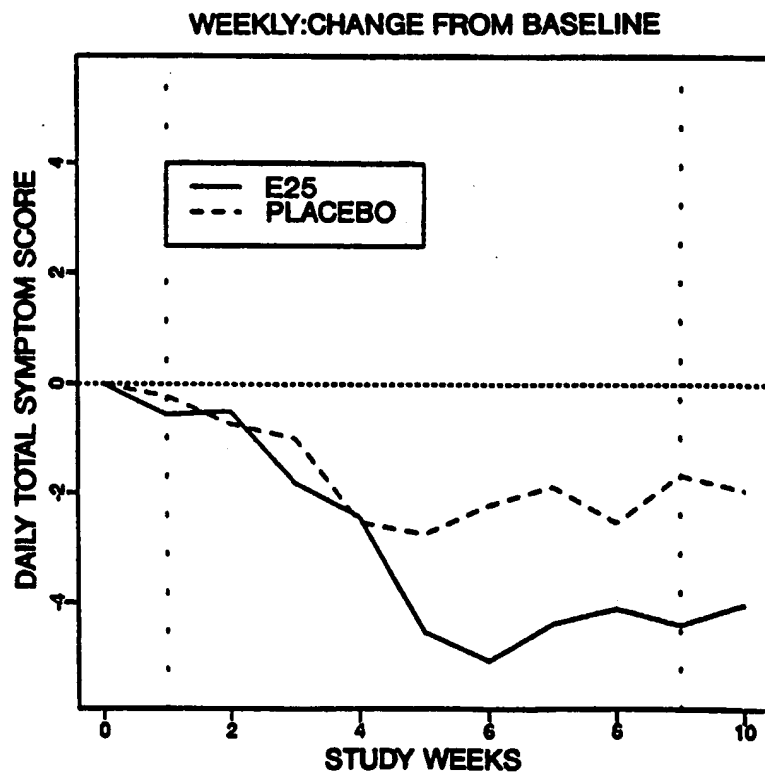


FIG.4B

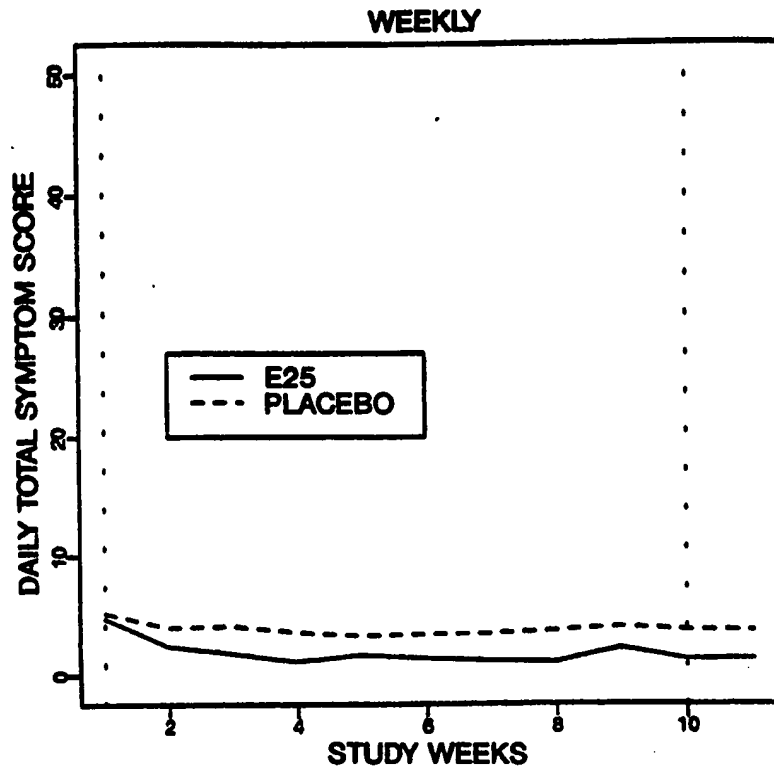


FIG.5A

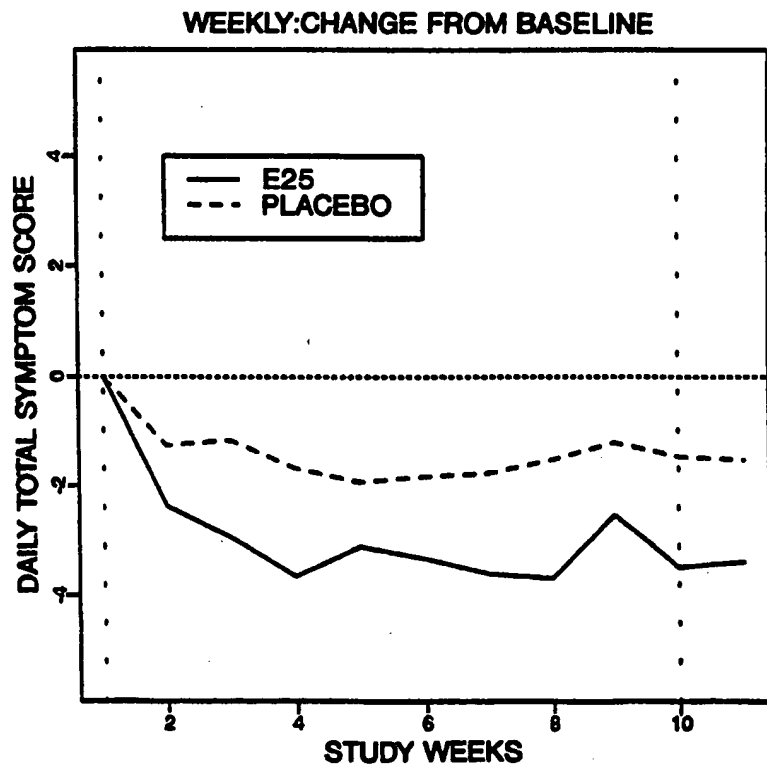


FIG.5B

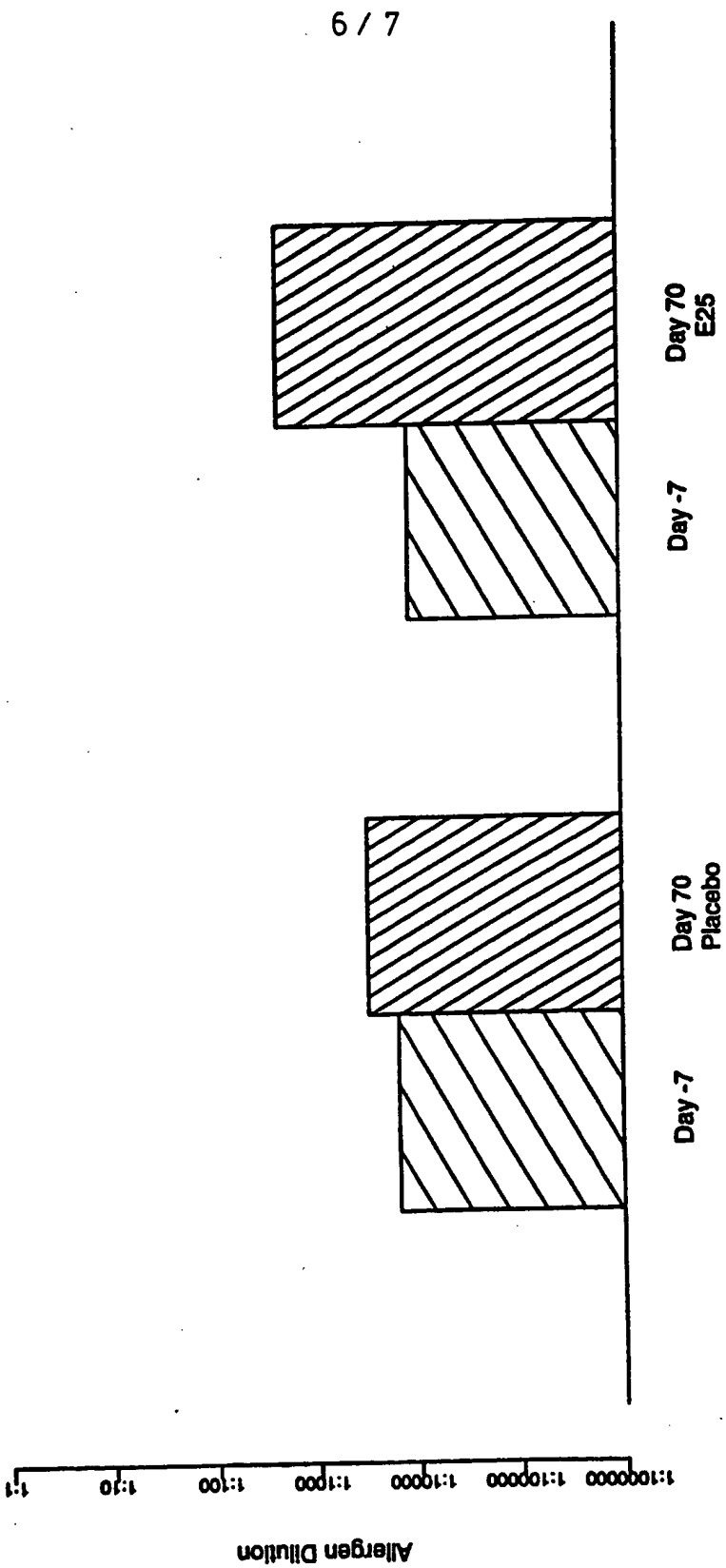
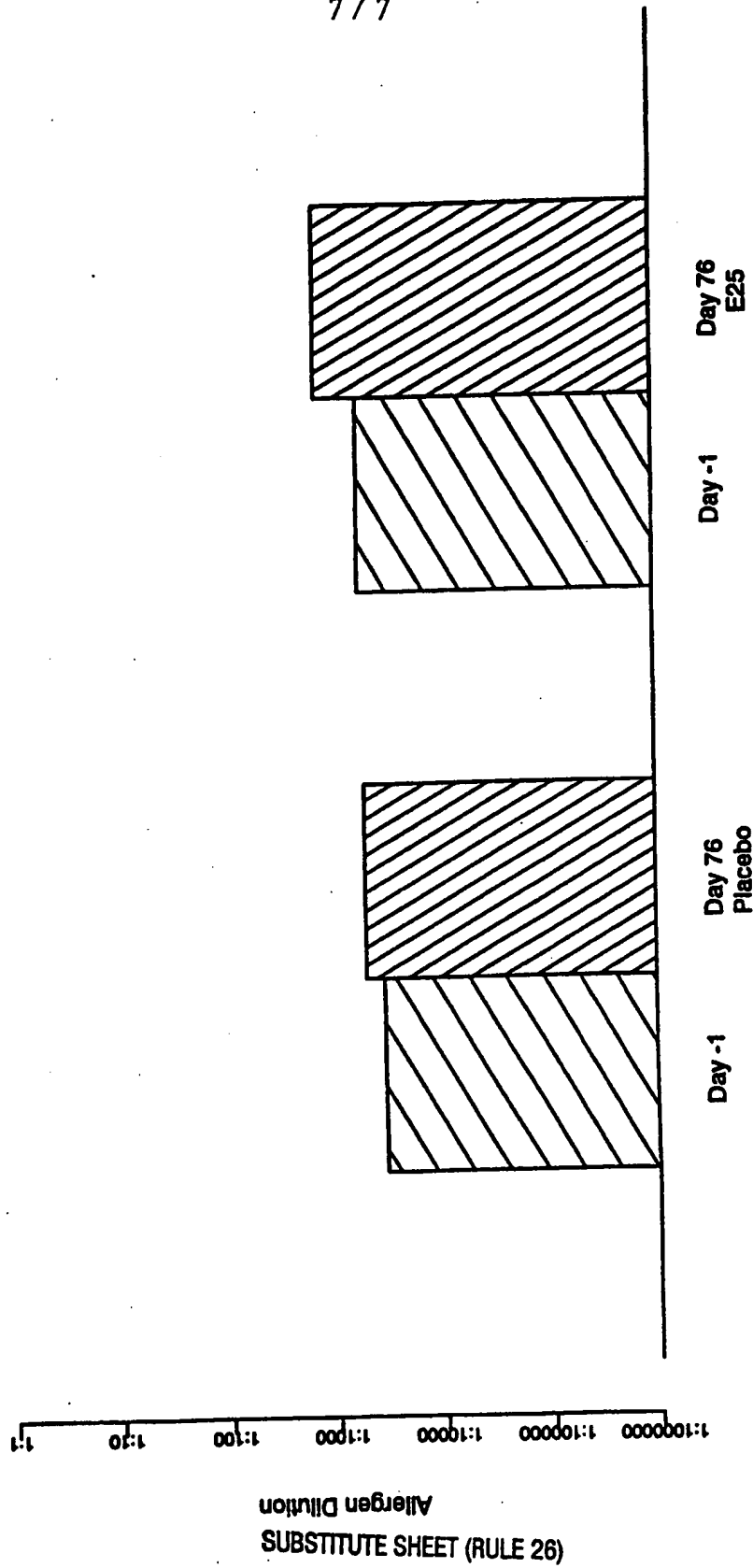


FIG.6

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/12275

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, vol. 95, no. 1 part 2, January 1995, ST. LOUIS, MO, USA, page 356 XP000611499 J. FROEHLICH ET AL.: "Multiple doses of a recombinant humanized monoclonal anti-IgE antibody are safely tolerated and decrease free serum IgE to undetectable levels." see abstract 863</p> <p style="text-align: center;">--- -/--</p>	1-5,7, 9-43

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

22 November 1996

Date of mailing of the international search report

02.12.96

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/12275

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, vol. 107, no. 1-3, May 1995, BASEL, SWITZERLAND, pages 308-312, XP000611560 R. SHIELDS ET AL.: "Inhibition of allergic reactions with antibodies to IgE." see the whole document ---	1-5,7, 9-43
X	THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, vol. 94, no. 5, November 1994, ST. LOUIS, MO, USA, pages 836-843, XP000611550 R. SABAN ET AL.: "Human FcERI-IgG and humanized anti-IgE monoclonal antibody MaE11 block passive sensitization of human and rhesus monkey lung." see abstract ---	1-5,7, 9-43
X	EP 0 648 499 A (C. RA & THE GREEN CROSS CORP.) 19 April 1995 see page 3, line 40 - line 43 see page 4, line 6 - line 26 ---	1-4,9, 11-43
X	EP 0 317 295 A (KISSEI PHARMACEUTICALS CO. LTD.) 24 May 1989 see page 2, line 1 - line 20 see claims ---	1-4, 11-43
X	WO 89 06138 A (TANOX BIOSYSTEMS) 13 July 1989 cited in the application see page 3, line 20 - page 6, line 19 see claims ---	1-6, 11-43
X	EP 0 589 840 A (CIBA GEIGY AG & TANOX BIOSYSTEMS INC.) 30 March 1994 see page 15, line 13 - page 16, line 2 ---	1-5,7, 9-43
X	BIO/TECHNOLOGY, vol. 8, February 1990, NEW YORK, NY, USA, pages 122-126, XP002019200 T. CHANG ET AL.: "Monoclonal antibodies specific for human IgE-producing B cells: a potential therapeutic for IgE-mediated allergic diseases." see discussion ---	1-5,9-43
	-/--	

INTERNATIONAL SEARCH REPORT

Int: nal Application No

PCT/US 96/12275

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, vol. 685, 23 June 1993, NEW YORK, NY, USA, pages 549-560, XP000611552 S. NAKAJIMA ET AL.: "Effect of saiboku-to (TJ-96) on bronchial asthma." see abstract see table 1</p> <p style="text-align: center;">---</p>	<p>1-4, 11-43</p>
A	<p>WO 93 19197 A (IMMUNET) 30 September 1993 see examples see claims</p> <p style="text-align: center;">---</p>	<p>8</p>
P,X	<p>THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, vol. 97, no. 1 part 3, January 1996, ST. LOUIS, MO, USA, page 315 XP000611551 D. COCKCROFT ET AL.: "rhuMAb-E25 (E25), humanized murine monoclonal anti-IgE, inhibits the allergen-induced early asthmatic response (EAR)." see abstract 532</p> <p style="text-align: center;">-----</p>	<p>1-5,7, 9-43</p>

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/ 12275

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-43
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although these claims are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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INTERNATIONAL SEARCH REPORT

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PCT/US 96/12275

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